Nitrosamine formation from different *Catha edulis* leaves extracts under simulated gastric condition

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Introduction

Fresh leaves of khat (Catha edulis Forsk) are customarily chewed to attain a state of stimulation. The facts that cathinone has a close structural similarity to amphetamine, and that both share common pharmacodynamic features, led to the conclusion that cathinone is the most important active ingredient of khat, which causes major pharmacological effects (1). The common adverse effects of khat are wide and variable (2, 3, 4). Among these is the suggestion that the high tannin content of the leaves is responsible for the observed gastritis and the apparently high prevalence of oesophageal carcinoma in Yemen (5).

Catha edulis leaves contain some primary amines, such as cathinone, cathine, and norephedrine (6) and secondary amines, such as ephedrine and pseudoephedrine (7), which may be considered to be precursors of nitrosamines (potent carcinogens) in the presence of nitrite. Significant concentrations of nitrite are present in the dietary intake added either as a preservative or formed by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates. The aim of the present study is to evaluate the formation of nitrosamine compounds from the nitrosation of the aqueous extracts of different types of Catha edulis leaves in vitro and in simulated gastric fluid.

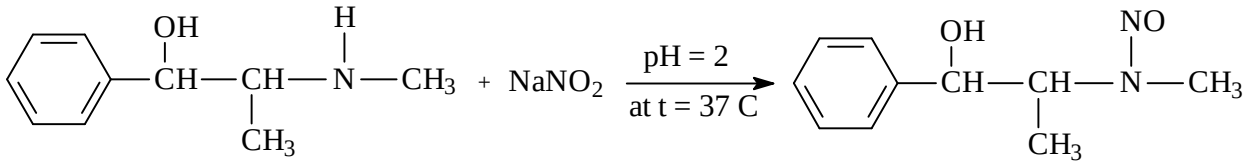
Materials and Methods

1. Materials

Five different types of Catha edulis leaves were collected (Sabri, Saotti, Ansi, Baladi and Hamdani) from different areas in Yemen namely Taiz, Hajah, Dhamar and Sana’a, respectively. All samples were air-dried and ground using a coffee grinder.

2. Methods

2.1. Nitrosation in aqueous solution with constant amount of sodium nitrite.



Ephedrine (Secondary amine) Nitrosoephedrine (Carcinogenic)

2.2. Nitrosation under simulated gastric conditions

Simulated gastric juice was prepared according to the method described by Gillat et al. (8).

2.3. Nitrosation of Catha edulis extracts in aqueous solution or under simulated gastric conditions with different amounts of sodium nitrite.

Reactions were carried out as described for aqueous nitrosation, but different amounts of sodium nitrite were added. Samples without addition of sodium nitrite were used as a blank.

2.4. Colorimetric determination of N-nitrosamines

Total apparent nitrosamine compounds were determined according to the method described by Alwan et al. (9).

Results and Discussion

The results in this study report total apparent nitrosamine compounds, expressed as nitrosoephedrine equivalents, since the latter was used as a reference in this study. The results of nitrosation products of aqueous extracts from different types of

Catha edulis leaves, with a constant concentration of $NaNO_2$, either in queous solution or under simulated gastric juice conditions, are shown in Table 1.

Table 1: Total apparent nitrosamine compounds (mg/100 g of Catha edulis as DM) formed by the addition of 1 g of sodium nitrite to different types of Catha edulis extracts, either in aqueous solution or in simulated gastric juice.

Type of Catha edulis	Total apparent nitrosamines as nitrosoephedrine[A]	
	In aqueous solution	In simulated gastric juice

Saotti	319 ± 44.6a	79.4 ± 11.5a
Baladi	300 ± 35.0a	55.0 ± 10.6b
Ansi	200 ± 0.01b	22.5 ± 5.3d
Sabri	200 ± 35.4b	23.1 ± 2.7d
Hamdani	93.8 ± 8.8c	35.6 ± 4.4c

Values (means ± SD) in the same column followed by the same letter are not significantly different at $P < 0.05$.
[A] Hot water extraction was used in the aqueous solution but not under the simulated gastric conditions.

The total apparent of nitrosamines obtained by nitrosation of Catha edulis leave extracts with constant concentration of $NaNO_2$ in simulated gastric juice were much lower than those obtained in aqueous solution and this could be attributed to the hot extraction with distilled water before the incubation, when the hot extraction may give higher amounts of extracted amines which, in turn, may convert to nitrosamines by nitrosation with $NaNO_2$. The results obtained by this treatment followed the same trend as those

obtained in aqueous solution nitrosation with the exception of the Hamdani (Table 1).

Table 2 shows, the amounts of total apparent nitrosamines obtained by nitrosation of aqueous extracts of the Sabri type of Catha edulis leaves using different concentrations of $NaNO_2$, either in aqueous solution or under simulated gastric juice conditions. The total apparent nitrosamines obtained from both treatments were directly proportional to the concentrations of $NaNO_2$. The results obtained in the aqueous solution nitrosation were much higher (4- to 6.5-fold) than those obtained in simulated gastric juice (Table 2), which can be attributed to the hot extraction, as explained earlier.

Table 2: Total apparent nitrosamine compounds (mg/100 g of Catha edulis as DM) formed by the addition of different amounts

of sodium nitrite to Sabri Catha edulis extract, either in aqueous solution or in simulated gastric juice

Amount of sodium nitrite added (mM)	Total apparent nitrosamines as nitrosoephedrine[A]	
	In aqueous solution	In simulated gastric juice
10	70.8 ± 5.8a	10.9 ± 2.7a
5	27.5 ± 5.8b	6.5 ± 1.2b
2.5	17.5 ± 3.7c	4.3 ± 0.7c
1	5.0 ± 1.1d	ND
0.5	ND	ND
0.1	ND	ND

0.05	ND	ND

Values (means ± SD) in the same column followed by the same letter are not significantly different at $P < 0.05$.
[A] Hot water extraction was used in the aqueous solution but not under the simulated gastric conditions.

The minimum amounts of $NaNO_2$ giving a detectable level of nitrosamines, either in aqueous solution or in simulated gastric juice, were 1 and 2.5 mM, respectively. The total apparent nitrosamines obtained from all types of Catha edulis used in our study, either in aqueous solution or simulated gastric juice, were much higher than that obtained from the nitrosation of tea made from the plant of Ephedra Altissima, either in vitro or in simulated gastric juice, as well as that obtained from Ephedra Foliata (10). The former study showed that nitrosamines, such as N-nitrosoproline, N-nitrosoephedrine, N- nitrosopseudoephedrine and N-nitrosomethylbenzylamine can be formed under the simulated gastric conditions using a constant nitrite concentration of 25 μM

(10), which is representative of the upper range in the normal acidic fasting stomach with continuous replenishment of nitrite from saliva (10). The nitrosamines formed due to nitrosation in the earlier studies have been implicated in the incidence of liver, lung, pharyngeal, esophageal and forestomach carcinomas (11).

In the light of the findings it seems that the pH in the gastro-esophageal junction and the cardia of the stomach favours nitrosation, as it escapes the buffering effect of food; also this anatomical site corresponds with increasing incidence of mutagenesis and carcinogenesis. This then raises the question of whether the observed high incidence of esophageal and forestomach carcinomas in Yemen could be attributed to the formation of nitrosamines in vivo from the secondary amines present in Catha edulis leaves.

Nitrate in food or drinking water is absorbed in the small intestine and about 25% of the intake is actively secreted by the salivary glands into the oral cavity, where approximately 20% of this fraction is converted into nitrite by oral bacteria. This nitrite gradually enters the stomach after swallowing of oral fluid where, at low pH, nitrite will be converted to nitrous acid which reacts with secondary amines to give nitrosamines. It is known that khat chewers consume large amounts of water during and after khat chewing (amount of nitrate in water = 80–125 mg/l), which means increased nitrate intake and, consequently, increased production of nitrite and nitrosamines.

Conclusion

In conclusion, the new findings of nitrosamines formation from Catha edulis leaves may be a likely explanation of the observed high incidence of esophageal and stomach carcinomas in Yemen, along with the high tannin content of Catha edulis leaves.

References

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